PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

M. Clark DALE et al. Attorney Docket No.: P66143US1

Serial No. 10/056,063 Art Unit: 1651

Filed: January 28, 2002 Examiner: Hebert J. Lilling

For: A HIGH SPEED, CONSECUTIVE BATCH OR CONTINUOUS, LOW EFFLUENT PROCESS FOR THE PRODUCTION OF ETHANOL FROM :MOLASSES, STARCHES OR SUGARS

DECLARATION

I, M. CLARK DALE, am one of the inventors of the above-identified application.

All strains of *Saccharomyces cerevisae* are characterized by the vigorous fermentative (oxygen limited) conversion of sugars (primarily glucose, sucrose, and fructose) to ethanol.

The Advisory Action of December 4, 2003, alleges that "there is no showing that the claimed limitations meet the conditions of being suitable intrinsic properties to allow the product microorganism." Indeed, those characteristics:

(i) has the capability of maintain a totally floc mode characterized by yeast pellets of 0.1 to 5 mm diameter in a fermentation medium,

(ii) shows a yeast-free cell (single or budding double cells) of less than 0.5 g/L with a yeast floc density as high as 100 g/L or higher, and

(iii) has a limiting osmo-tolerance for ethanol productivity of about 5.0 os/kg

are truly intrinsic properties of the BPSC-15 floc yeast which distinguish it from all other strains of *Saccharomyces cerevisae* (as well as any other yeast strain to Applicants' knowledge).

In somewhat expanded details, these clearly inherent characteristics consist of:

1) the inherent characteristic of the BPSC-15 yeast strain to stay in a self flocculent mode, with large coherent, stable pellets (even when conditions for growth and/or fermentation are optimal i.e.high sugar and nutrients, low ethanol). This is a rare characteristic. Many *S. cerevisae* strains tend to floc and fall to the bottom of the fermenter slowly (24-60 hours) at the completion of a fermentation when sugar is exhausted and ethanol levels are high. The BPSC-15 strain, however, maintains a stable floc pellet at all times, even when exposed to conditions favorable for cell growth (high sugar and nutrient concentrations and low ethanol concentrations). The BPCS-15 flocs settle quickly (30 seconds to 5 minutes) in a fermentation broth any time that agitation of the fermenter is stopped. This property allows a very high floc cell density to be maintained in a variety of bio-reactor configurations; leading to high speed fermentations; and reduced capital costs for fermentation tanks, or a greatly expanded production capacity with fermentation vessels in existing ethanol plants. A further advantage of the flocc charactristic is the production of a valuable yeast 'paste' by-product without need for centrifuging.

2) the inherent characteristic of the BSC-15 yeast strain to release very few 'free cells' into the fermentation media, where free cells are defined as single or double 'budding' cells which stay suspended in an active fermentation. This characteristic of staying almost entirely in the flocculent mode results in fermentation broths which are quite clear [having a 'free cell density' as measured by cell count or Optical Density (OD) of under 0.5 g dry weight yeast per Liter]. This contrasts with standard *S. cerevisae* strains which usually reach a free suspended cell density of between 5 and 15 g/L free cells prior to flocculation. OD's of BPSC-15 fermentation broth were measured at between 0.2 to 0.8 OD (corresponding to a free cell density of between 0.05 to 0.19 g/L)over a 23 hour fermentation period versus OD's of 3.6 to 23 OD (corresponding to 0.9 to 5.5 g/L) for a standard *S. cerevisae* strain (CBS 2959) over a 40 hour fermentation period, as described in Dale's Declaration of November 6, 2003.

3) The inherent characteristic of the BPSC-15 strain to be able to ferment sugars to ethanol in a fermentation broth with a solution osmolality of up to 5.0 os/kg. This compares with most *S. cerevisae* having a limiting tolerance of 3.0 os/kg. This osmo-tolerance characteristic allows the fermentation of molasses or corn sugars with a high degree of stillage backset, leading to reduced amounts of stillage generated and thus reducing costs for waste water treatment/evaporation.

Each of the characteristics, (i), (ii) and (iii), is an intrinsic characteristic by itself. The combination of all three of these intrinsic characteristics for a strain of *Saccharomyces cerevisae* truly defines subject matter which is separate and distinct from what has been previously disclosed in this art.

In the noted Advisory Action molecular weight and density are cited as exemplary "intrinsic" properties. To one of ordinary skill in the subject art, the properties set forth in the Applicants' claim 1 are equally, if not more, intrinsic in the context in which they are presented.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 23rd day of January, 2004.

M. Clark Dale

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